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# Chemical Interactions between Leachables and Biopharmaceuticals

## COMBINED IN-SILICO AND EXPERIMENTAL MODEL (WITH INSULIN AS A MARKER COMPOUND) TO MONITOR THE POTENTIAL IMPACT ON QUALITY AND SAFETY OF THERAPEUTIC PROTEINS

*Paulo Forte*
*Study Director*
*Extractables & Leachables*

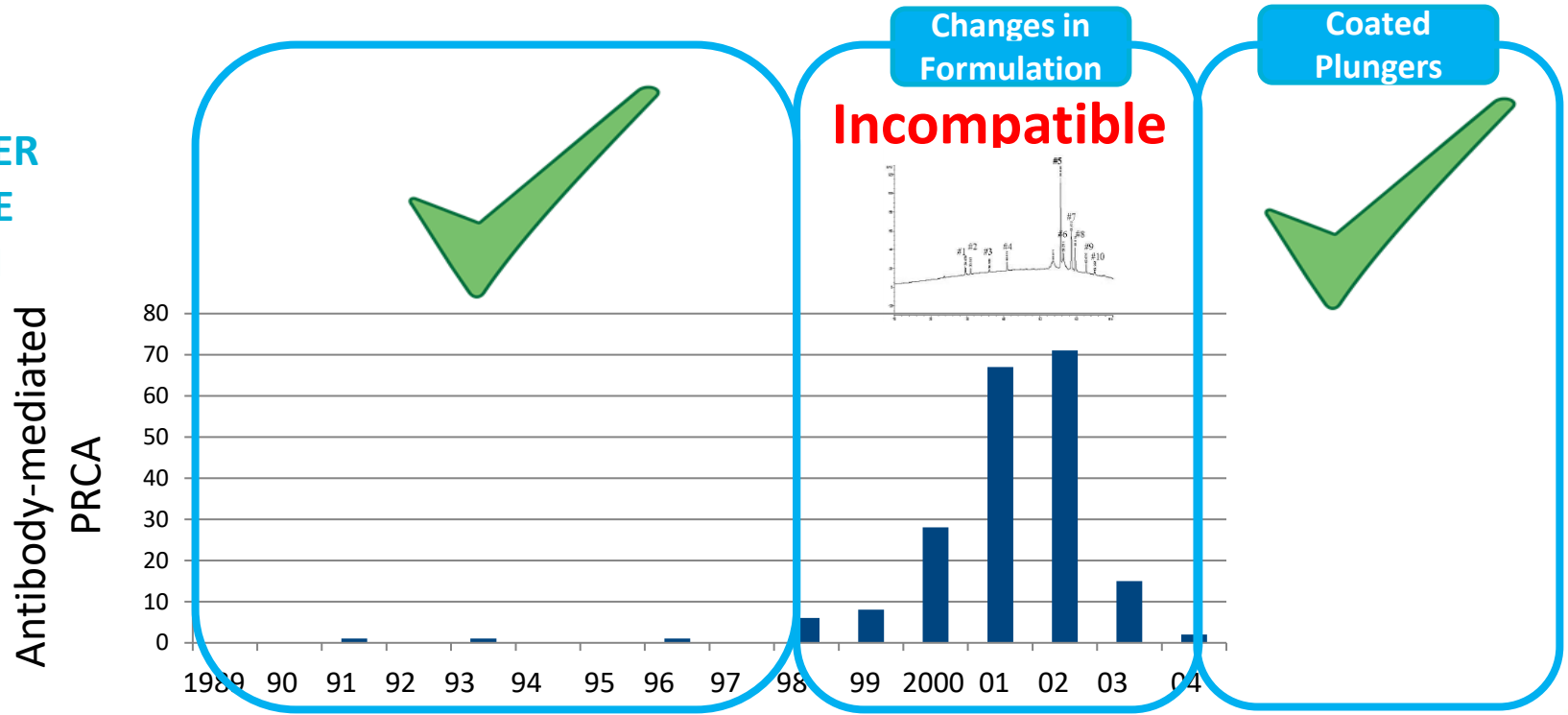
# Eprex case


- Eprex = Human Recombinant EPO
- Introduced in late ‘80 early ’90 – Janssen Cilag
- Increase Red Blood Cell-count in Chronic Kidney Disease Patients

- Until ’98: no side effects
- From ‘98 onwards: increased incidence of PRCA
  - *Caused a drop in Hematocrit (instead of an increase)*
  - *Immune response*

# Eprex case: Incidents of AB-mediated Pure Red Cell Aplasia

**CONTAINER CLOSURE SYSTEM**




Red Blood Cell levels substantially reduced because of an Anti-body mediated immune response

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Basant Sharma[1], PhD; Fred Bader[1], PhD; Tom Templeman[1], PhD; Peter Lisi[2], PhD; Mary Ryan[3], PhD; George A. Heavner[4], PhD

# Eprex case

| Peak[a] | Compound | Average concentration[b] |
|---|---|---|
| 1 | Unknown | Unknown |
| 2 | Bisphenol A | 0.070 |
| 3 | 4-*tert*-amylphenol | 0.046 |
| 4 | 2-chloro-4-*tert*-amylphenol | 0.037 |
| 5 | Vultac® 2 disulfide | 0.778 |
| 6 | 2,2'-methylene-bis-4-*tert*-amylphenol | 0.243 |
| 7 | Vultac® 2 trisulfide | 0.235 |
| 8 | Vultac® 2 tetrasulfide | 0.142 |
| 9 | Vultac® 2 pentasulfide | 0.063 |
| 10 | Vultac® 2 hexasulfide | 0.024 |

QUESTION: Who could have **predicted an anti-body mediated immune response**, based upon those analytical data?

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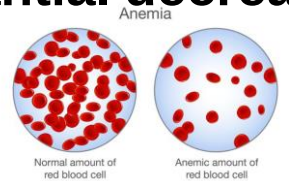
Basant Sharma[1], PhD; Fred Bader[1], PhD; Tom Templeman[1], PhD; Peter Lisi[2], PhD; Mary Ryan[3], PhD; George A. Heavner[4], PhD

# Mode of action - Hypothesis in the early work:

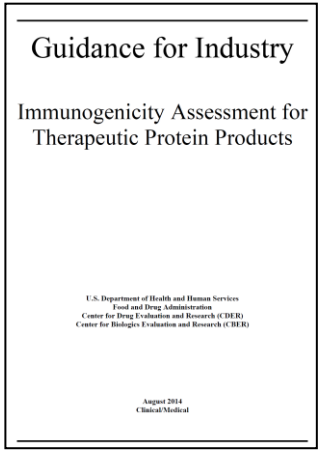
**Leachables** (one or more) could have **adjuvant-like properties**, "boosting" an immune response, which is **causing ADA's (Anti-Drug-Antibodies) to be formed**

**ADA's attacked both endogenous & exogenous EPO**
ultimately resulting in **a substantial decrease of Red Blood Cells (PRCA/Anemia)**




However, the **"adjuvant like properties"** of the detected compounds were studied in **animal models**, but **no ADA's were observed**.

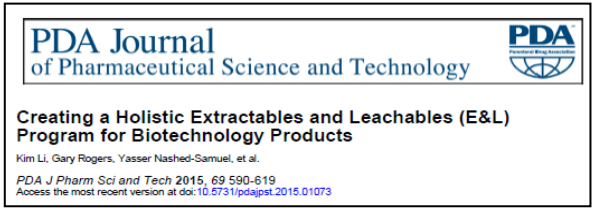
# Mode of action – New Line of Thinking:


Guidance for Industry

Immunogenicity Assessment for Therapeutic Protein Products

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

August 2014
Clinical/Medical


PDA Journal of Pharmaceutical Science and Technology

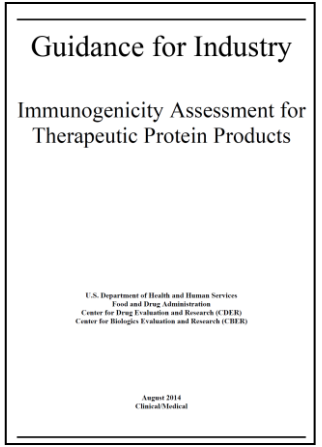
**Creating a Holistic Extractables and Leachables (E&L) Program for Biotechnology Products**

Kim Li, Gary Rogers, Yasser Nashed-Samuel, et al.

*PDA J Pharm Sci and Tech* 2015, 69 590-619
Access the most recent version at doi:10.5731/pdajpst.2015.01073


**Reactive Leachables** may form **covalent bonds** with Biologics and this may lead to **Immuno Responses**

# Relevance of reactive leachables


Guidance for Industry

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August 2014
Clinical/Medical

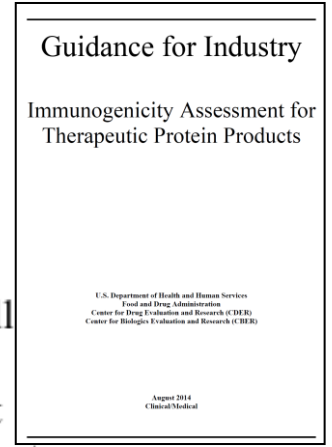
## 8. *Container Closure Considerations*

Leached materials from the container closure system may be a source of materials that enhance immunogenicity, either by chemically modifying the therapeutic protein product or by having direct immune adjuvant activity, including the following:

- Organic compounds with immunomodulatory activity may be eluted from container closure materials by polysorbate-containing formulations: a leachable organic compound involved in vulcanization was found in a polysorbate formulated product when the stopper surfaces were not Teflon coated (Boven et al. 2005). → Refers to Eprex case

# FDA Guidance for Industry (2014) Immunogenicity

## Risk evaluation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Clinical/Medical

*Recommendations*

Whenever possible, sponsors should obtain detailed information regarding a description of all raw materials used in the manufacture of the container closure systems for their products. Sponsors should conduct a comprehensive extractables and leachables laboratory assessment using multiple analytical techniques to assess the attributes of the container-closure system that could interact with and degrade protein therapeutic products.

Testing for leachables should be performed on the product under stress conditions,[9] as well as under real-time storage conditions, because in some cases the amount of leachables increases dramatically over time and at elevated temperatures. Product compatibility testing should be performed to assess the effects of container closure system materials and all leachables on product quality.

# FDA Guidance for Industry (2014) Immunogenicity – Therapeutic Proteins

... Conduct E/L assessment with multiple techniques
to assess the attributes of the C/C system that
**could interact with/degrade the protein therapeutic product**...


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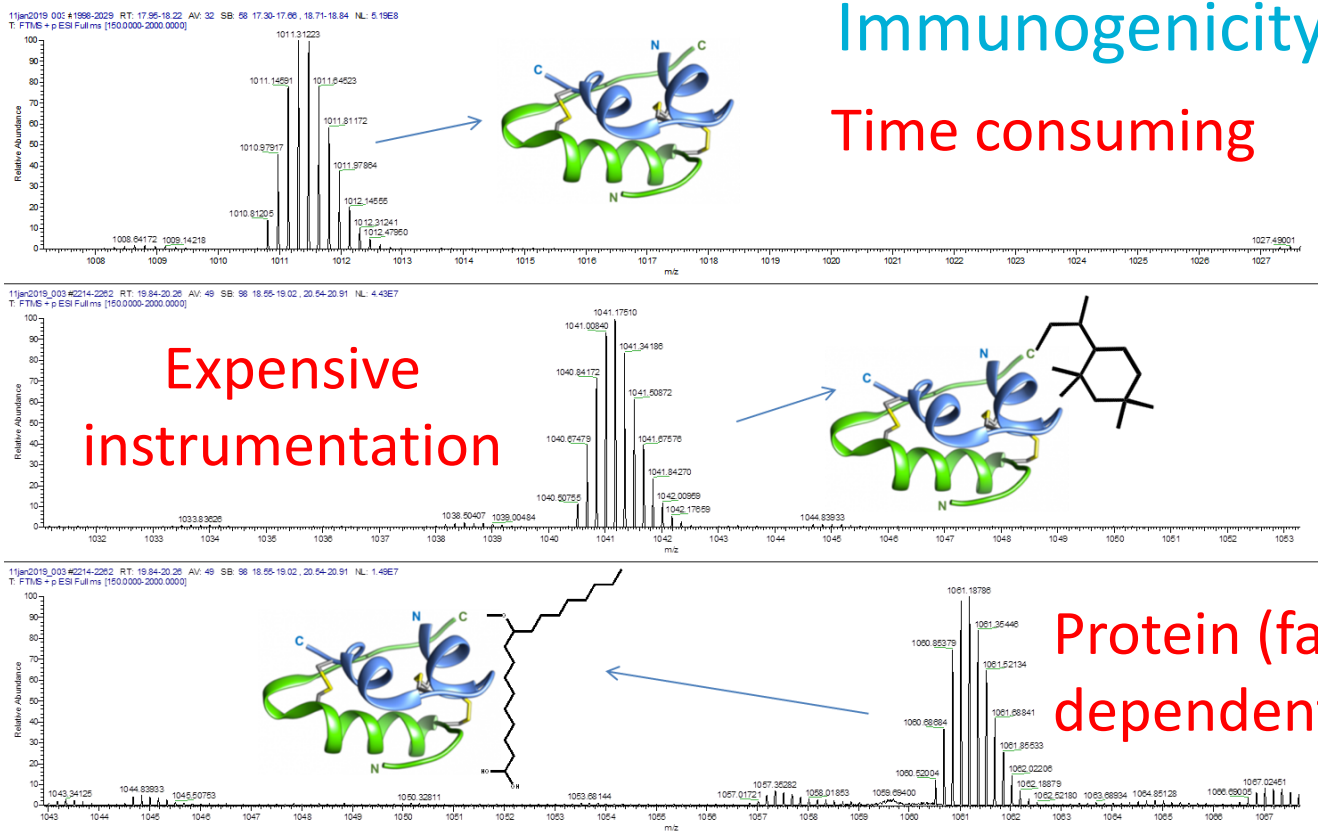
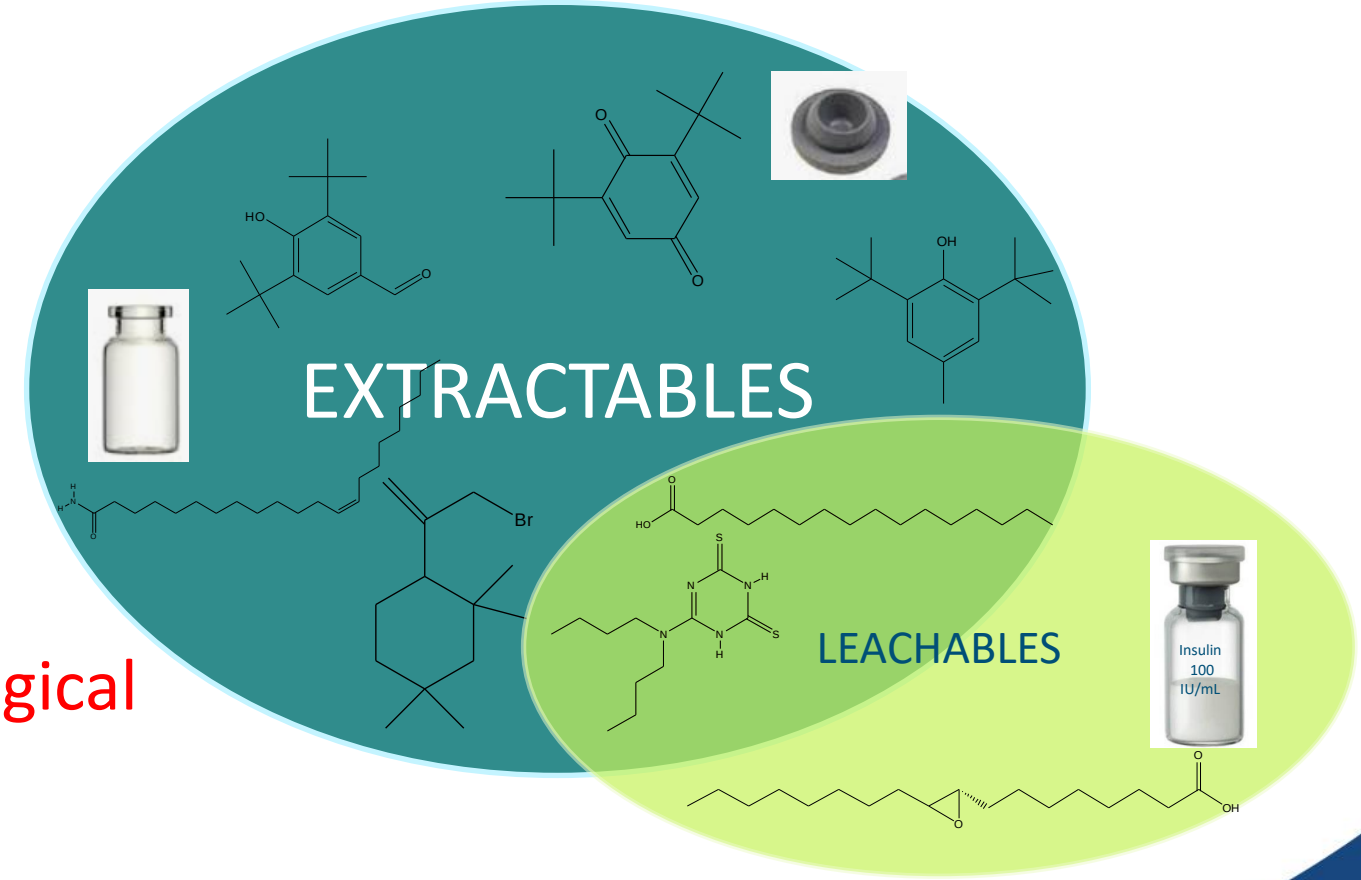
# Immunogenicity risk evaluation in E&L – how?



# Immunogenicity risk evaluation in E&L – how?


Initial Toxicological evaluation

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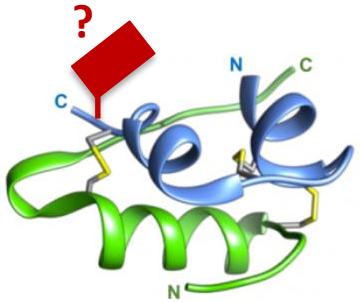
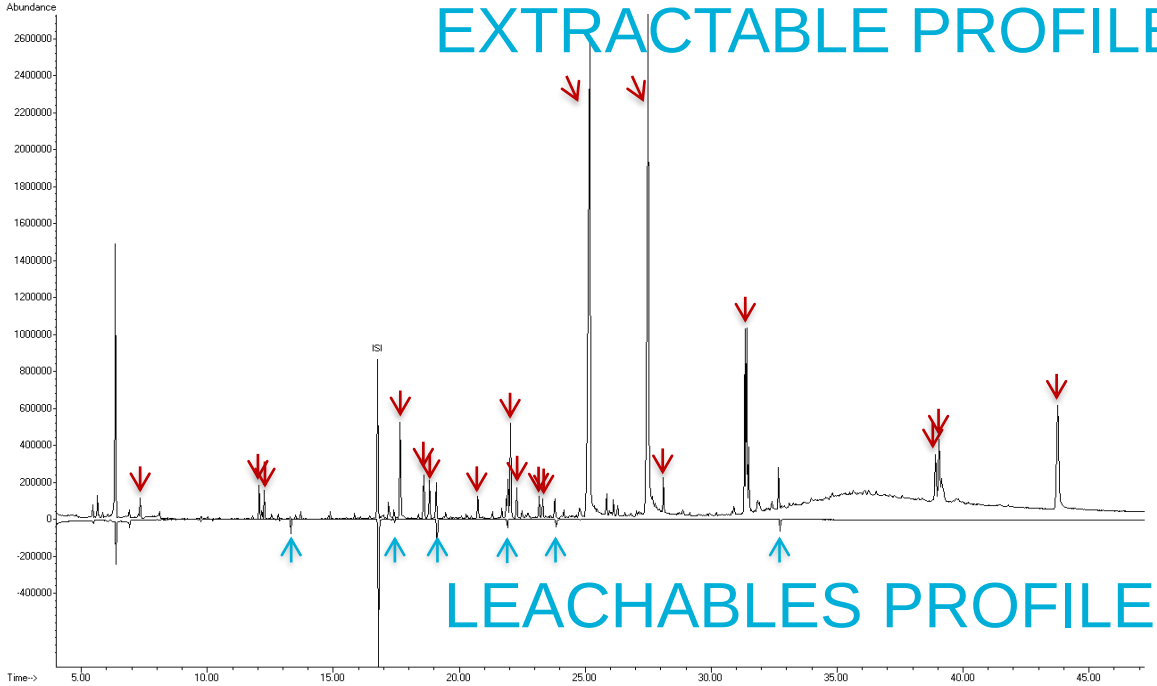
# Immunogenicity risk evaluation in E&L – how?


EXTRACTABLE PROFILE




LEACHABLES PROFILE

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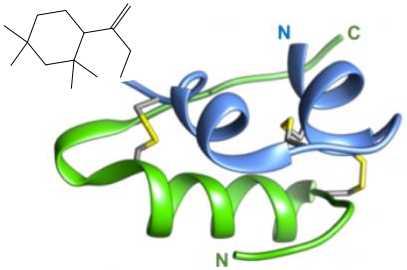
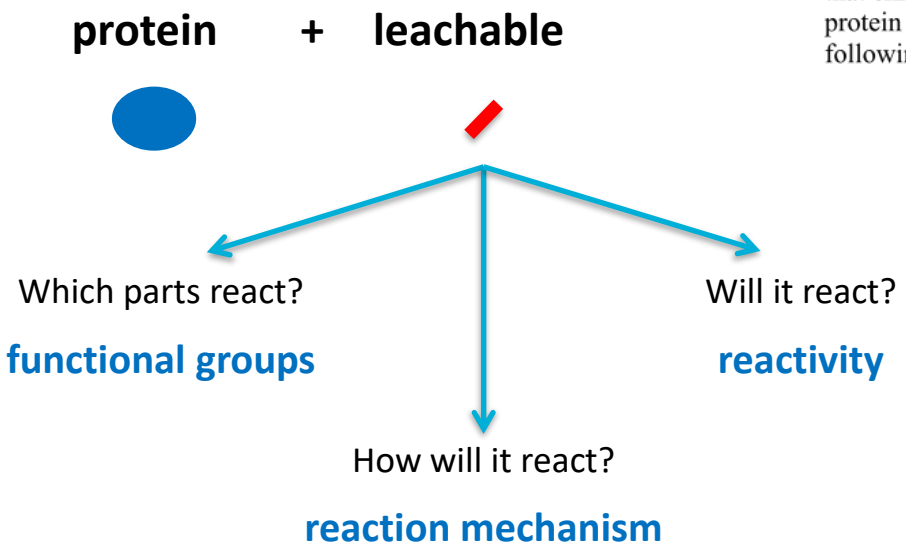
# QUESTION 1:

# CAN THE REACTIVITY OF LEACHABLES WITH PROTEINS AND PEPTIDES BE PREDICTED BY AN IN SILICO MODEL?



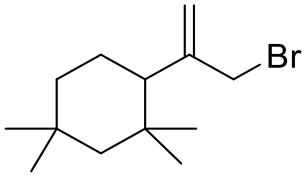
# Can we predict reactive leachables?



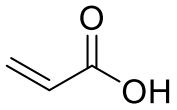

Leached materials from the container closure system may be a source of materials that enhance immunogenicity, either by chemically modifying the therapeutic protein product or by having direct immune adjuvant activity, including the following:

(FDA guidance 2014)

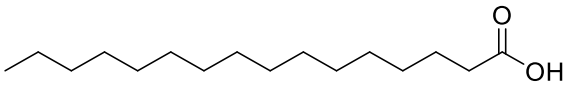
# *in silico* – reactivity (models based on)




$C_{13}H_{23}Br$ – rubber oligomer




acrylic acid




palmitic acid

| **glutathione**<br>potency by GSH | **lysine**<br>DPRA (13%) Lys | **cysteine**<br>DPRA (13%) Cys |
|---|---|---|
| extremely reactive<br>highly reactive | reactive | reactive |
| moderately reactive | grey zone | grey zone |
| slightly reactive<br>suspect | unreactive | unreactive |

*in silico* prediction

QSAR TOOLBOX

**NELSON LABS**

high reactivity

moderate reactivity

low reactivity

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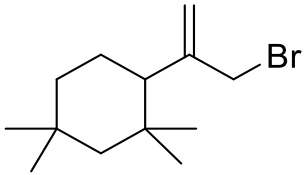
# *in silico* – reactivity (models based on)

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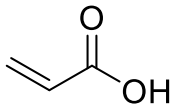
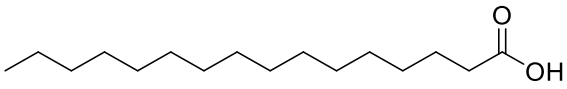



acrylic acid

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*in silico* prediction

QSAR TOOLBOX

**NELSON LABS**

high reactivity

moderate reactivity

low reactivity

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| CAS | Name | Nels eactivity |
|---|---|---|
| [illegible] | palmitic acid | |
| 84-66-2 | diethyl phthalate | |
| 5323-65-9 | 2-chloro-4-tert-amylphenol | |
| | vultac 3 | |
| 96-76-4 | 2-4-di-tert-butylphenol | |
| 78-42-2 | tris(2-ethylhexyl) phosphat | |
| 93-89-0 | ethyl benzoate | ow |
| 95-47-6 | o-xylene | low |
| 96-37-7 | methylcyc | low |
| 78-59-1 | alp | moderate |
| 80-56-8 | | moderate |
| 544-10-5 | | moderate |
| 1120-7 | | moderate |
| | | moderate |
| | acid | high |
| | oligomer | high |
| | etophenone | high |
| | orone diisocyanate | high |
| | styrene oxide | high |
| 85 | phthalic anhydride | high |
| 80- | methyl methacrylate | high |

AT NELSON LABS:
REACTIVITY ASSESSMENT OF OVER 3500 COMPOUNDS

# QUESTION 2:

# CAN THE PREDICTED REACTIVITY OF LEACHABLE COMPOUNDS BE OBSERVED IN BIOLOGIC DRUG PRODUCTS?

# Wet lab experiments: model system


## Why Lyophilized Insulin (Glargine)?

- **High relevancy** because of widespread use and increasing delivery device market
- **Lyophilized** Insulin is **readily available** as reference material (EDQM)
- **Acceptable analytical complexity**

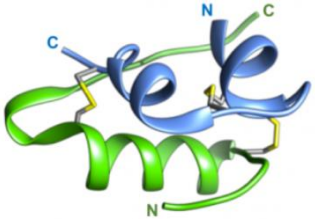
# Wet lab experiments: leachables


| Leachable | In silico prediction? | Reacted? |
|---|---|---|
| Palmitic acid | Low reactivity | |
| Diethyl phthalate | Low reactivity | |
| 2-chloro-4-tert-amylphenol | Low reactivity | ? |
| Vultac 3 | Low reactivity | |
| Caprolactam | Low reactivity | |
| 7,9-di-tert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione | Low reactivity | |

| Leachable | In silico prediction? | Reacted? |
|---|---|---|
| *cis*-9,10-epoxystearic acid | High reactivity | |
| $C_{13}H_{23}Br$ rubber oligomer | High reactivity | |
| 2-Chloroacetophenone | High reactivity | ? |
| Isophorone diisocyanate | High reactivity | |
| 2,6-di-tert-butyl-p-benzoquinone | High reactivity | |
| Dioctyl disulfide | High reactivity | |

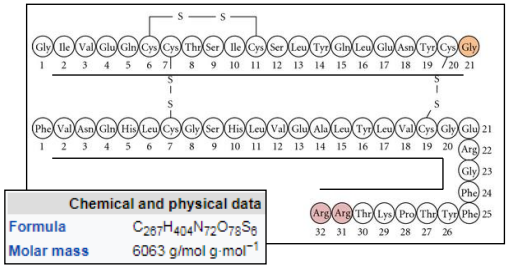
# Wet lab experiments: overview

**Insulin Glargine** + **leachables**




Which parts react?

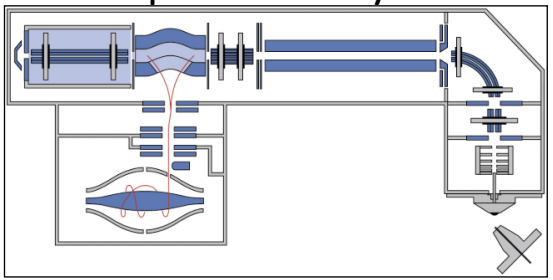
**Structure Insulin Glargine**




How to analyze?

**Instrumentation**

Q-Exactive Orbitrap
Liquid Chromatography
Mass Spectrometry



How to prepare?

**Sample preparation**

Lyophilized Insulin is spiked with leachables
Solvent dried off, incubation 4 days @ 40°C

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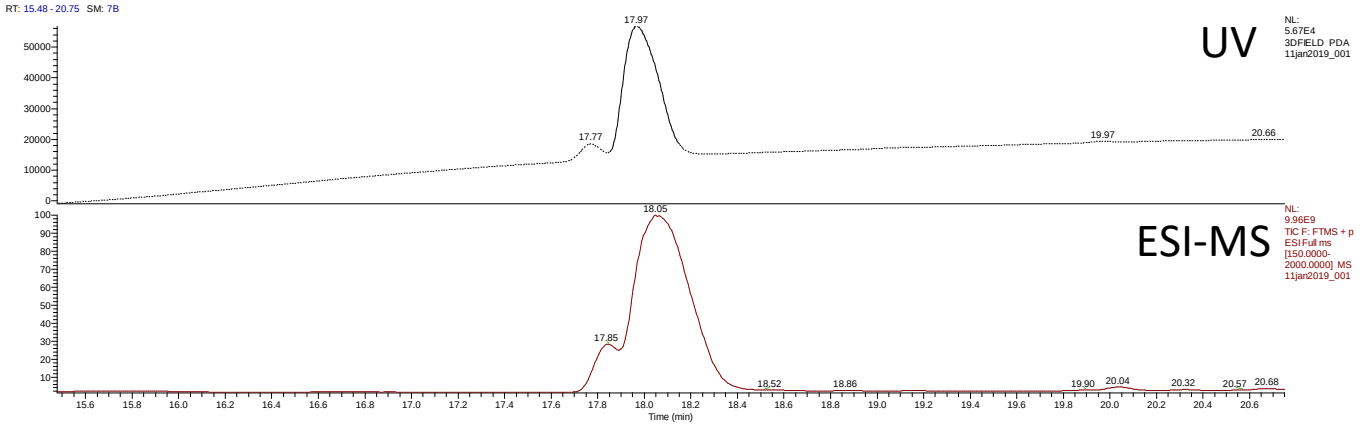
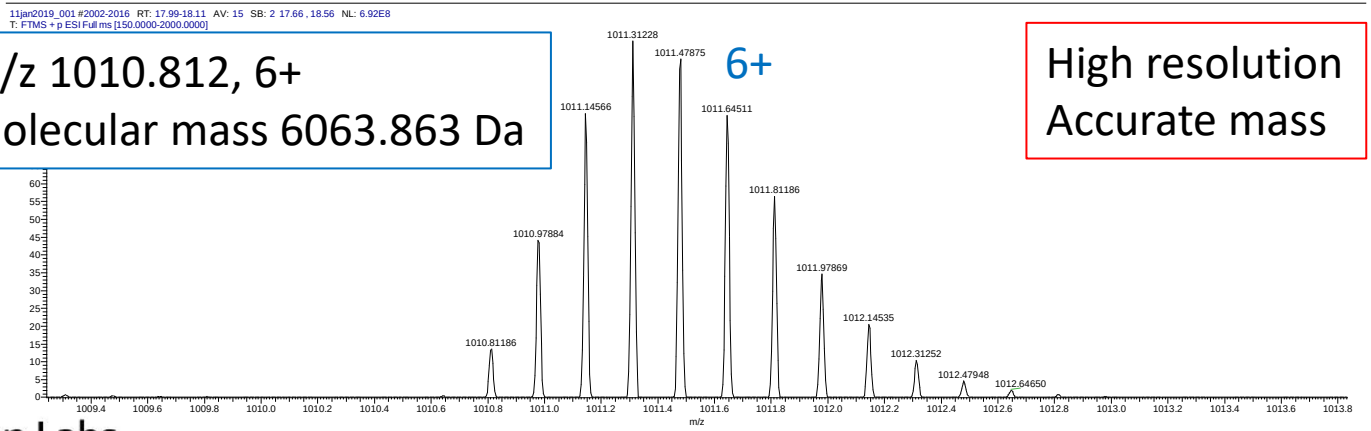
# Insulin Glargine Blank reference solution




m/z 1010.812, 6+
Molecular mass 6063.863 Da

6+

High resolution
Accurate mass

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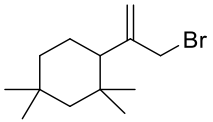
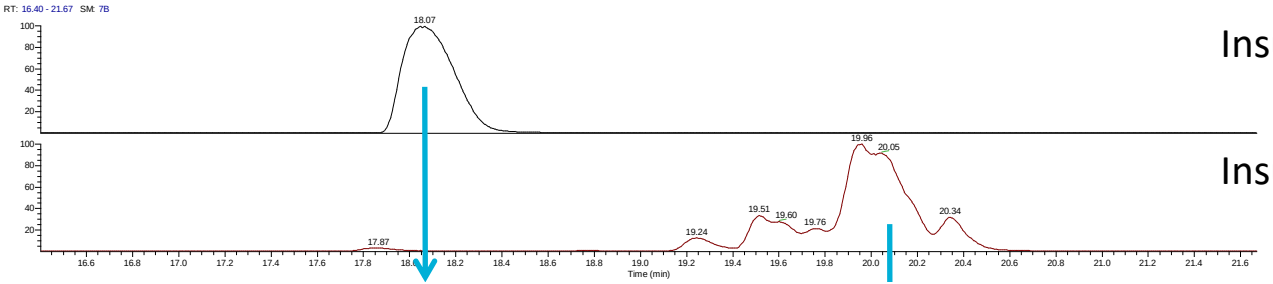
# Insulin Glargine + leachables (1)



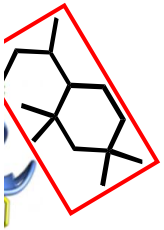




Insulin Glargine Blank

Insulin Glargine Adduct

Molec

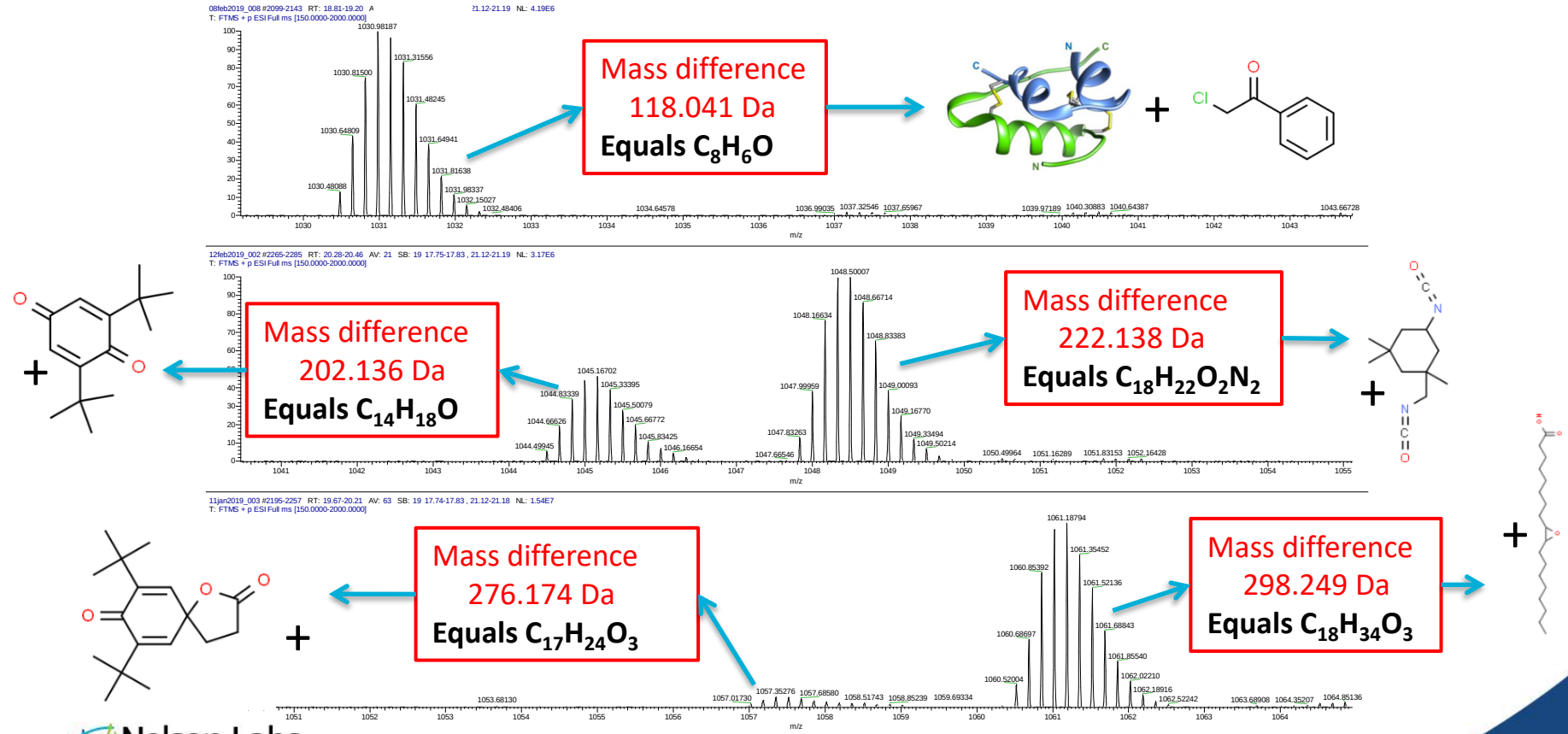
Molecu

m/z

N

# Insulin Glargine + leachables (2)



# Wet lab experiments: result overview


| Leachable | In silico prediction? | Reacted? |
|---|---|---|
| Palmitic acid | Low reactivity | ✗ |
| Diethyl phthalate | Low reactivity | ✗ |
| 2-chloro-4-tert-amylphenol | Low reactivity | ✗ |
| Vultac 3 | Low reactivity | ✗ |
| Caprolactam | Low reactivity | ✗ |
| 7,9-di-tert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione | Low reactivity | ✓ |

*Oxaspiro: low reactivity predicted in OECD toolbox*
*However: OECD toolbox also indicated a Michael Addition as a potential reaction mechanism, which was observed through a low reactivity*

| Leachable | In silico prediction? | Reacted? |
|---|---|---|
| *cis*-9,10-epoxystearic acid | High reactivity | ✓ |
| $C_{13}H_{23}Br$ rubber oligomer | High reactivity | ✓ |
| 2-Chloroacetophenone | High reactivity | ✓ |
| Isophorone diisocyanate | High reactivity | ✓ |
| 2,6-di-tert-butyl-p-benzoquinone | High reactivity | ✓ |
| Dioctyl disulfide | High reactivity | ✗ |

*Dioctyl Disulfide: HIGH reactivity predicted in OECD toolbox*
*However: OECD tests were performed in a reducing environment*
*Creating sulfide radicals that are very reactive.*
*Our testing was not performed under such conditions...*

# How to include in a leachable study?

extractable study
find potential leachables

initial risk assessment

leachables study

**PREDICT TOXICITY**

**NELSON's FIT SCREEN**
**(Fast Initial Toxicological *QSAR* Screening)**

supports material choice

indication of potential
toxicological risk to patient **– direct risk**

**PREDICT REACTIVITY**

**Additional**

supports material choice

get compounds for stability study **– direct risk**

indication of potential
immunogenic risk to patient **– indirect risk**

For Medical Device E/L: this tool could be used as a predictor for sensitizing properties

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# Conclusion

***In silico* prediction of reactivity**:

- cost effective with over 3500 chemical structures assessed on their reactivity
- Understanding the risk of reactive leachables can assist in
  - Optimizing Stability Studies
  - Risk mitigation for Immunogenicity
  - Material Selection

CONTAINER CLOSURE SYSTEM COMPATIBLE?

# THANK YOU

I would like to thank the following people who all were involved in the scientific work & interpretation:

- Dr. Piet Christiaens
- Dr. Ank Reumer
- Ruud Cuyvers
- Dr. Philippe Verlinde
- Dr. Lise Vanderkelen

# Thank you

# Questions?

InfoEurope@nelsonlabs.com
+32 16 40 04 84

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